MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Chemical Profiling and Evaluation of Antioxidant and Antimicrobial Properties of *Artemisia monosperma* Essential Oil

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Received:6/9/2025
Accepted:7/10/2025

Abstract: This study investigates the phytochemical composition, antioxidant, and antimicrobial properties of the crude methanolic extract from *Artemisia monosperma*, a desert plant traditionally used in folk medicine. Qualitative screening revealed the presence of diverse secondary metabolites, including abundant flavonoids, phenols, and tannins. Quantitative analysis confirmed high concentrations of total phenolics (142.57 ± 2.52 mg GAE/g), flavonoids (46.33 ± 2.10 mg CE/g), and tannins (27.62 ± 1.52 mg TAE/g). The extract demonstrated significant, dose-dependent antioxidant activity in the DPPH radical scavenging assay, with an IC_{50} value of 31.57 mg/L, attributable to its rich phenolic content. Furthermore, the extract exhibited potent broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens at a concentration of 10 mg/mL. Notably, it was effective against a multi-drug resistant Salmonella typhimurium strain (10.74 mm inhibition zone), against which standard antibiotics like ampicillin, chloramphenicol, and gentamicin showed no activity. Its performance against other bacteria, such as *Escherichia coli* (21.91 mm) and *Pseudomonas aeruginosa* (14.76 mm), was comparable or superior to several conventional antibiotics. The findings underscore that *A. monosperma* is a rich source of bioactive compounds with strong antioxidant and antimicrobial properties, validating its ethnomedicinal use and highlighting its potential as a natural alternative for pharmaceutical and therapeutic applications.

keywords: *Artemisia monosperma;* phytochemicals; DPPH; antimicrobial; multi-drug resistance.

1. Introduction

Medicinal and aromatic plants are a major source of bioactive compounds with wide therapeutic applications. Essential oils, in particular, represent complex mixtures of terpenes, phenolics, and oxygenated derivatives, which have been extensively studied for their antioxidant and antimicrobial activities [1].

Artemisia monosperma Delile (Asteraceae) is a perennial shrub native to arid and semi-arid regions of North Africa and the Middle East. Traditionally, it has been employed in folk medicine for the treatment of spasms, infections, and inflammatory conditions [2]. Phytochemical investigations of the genus *Artemisia* have revealed the presence of monoterpenes, sesquiterpenes, flavonoids, and phenolic compounds, many of which are associated with significant pharmacological properties [3-5].

Several studies have highlighted that the essential oil of *A. monosperma* is rich in monoterpene hydrocarbons such as α-pinene, β-pinene, and limonene, in addition to oxygenated sesquiterpenes like spathulenol, all of which contribute to its bioactivity [1,2]. Antioxidant assays demonstrated strong radical scavenging activity, with DPPH IC_{50} values reported around 5.3 mg/L, confirming its potential role as a natural antioxidant [6]. Moreover, antimicrobial evaluations revealed significant inhibitory effects against pathogenic bacteria and fungi, with minimum inhibitory concentrations (MICs) ranging between 0.5–2.5 µL per disc [7].

Given the urgent demand for novel natural antioxidants to combat oxidative stress-related diseases, as well as alternative antimicrobial agents in response to antibiotic resistance, *A. monosperma* essential oil presents a promising candidate for further research. Therefore, the current study aims to (i) chemically profile the extract of *A. monosperma* and (ii) evaluate its antioxidant and antimicrobial activities, thereby providing new insights into its potential pharmaceutical, food, and cosmetic applications.

2. Materials and Methods

2.1. Collection of Plant samples

Fresh aerial parts of *Artemisia monosperma* Delile were harvested during the flowering phase in May 2023 from the northwestern Desert of Egypt. Taxonomic identification and authentication were carried out following the keys of Boulos [8]. A voucher specimen (No. [Mans.00100125478]) was deposited in the Herbarium of Botany Department/Faculty of Science, Mansoura University. The collected material was carefully cleaned, shade-dried at ambient temperature (25 ± 2 °C) for 7–14 days and subsequently pulverized into fine powder with a mechanical grinder. The powdered samples were preserved in airtight containers at 4 °C until extraction and further analyses.

2.2. Qualitative phytochemical screening

Preliminary phytochemical analysis of the powdered aerial parts of *Artemisia monosperma* was conducted to detect major classes of secondary metabolites using standard methods [9,10] (Harborne, 1998; Trease & Evans, 2002) with minor modifications. Alkaloids were confirmed by Mayer's and Dragendorff's tests through creamy-white or reddish-brown precipitates, while flavonoids were identified by the Shinoda test, producing pink to red coloration. Phenols and tannins were revealed by the ferric chloride assay, yielding blue-black and greenish colors, respectively.

Saponins were verified by the frothing test, indicated by stable foam formation, and glycosides by the Keller–Killiani test, showing a reddish-brown ring at the interface. Terpenoids were detected by the Salkowski reaction, marked by a reddish-brown coloration, whereas steroids were confirmed through the Liebermann–Burchard test, producing green to blue hues. Anthraquinones were screened using Borntrager's test, which gave a pink to red coloration after ammonia treatment. All assays were performed in triplicate, and outcomes were qualitatively recorded as present or absent..

2.3. Quantitative phytochemical

2.3.1. Total tannin contents

The tannin contents were analyzed following the procedure of vanillin-hydrochloride assay [11], in which the absorbance of the sample was measured after treatment with freshly prepared vanillin-hydrochloride. The attained values of tannin contents of for the extracted plant samples were articulated as gram tannic acid equivalents / 100-gram dry plant. The capacity of tannins of the investigated samples was calculated from tannic acid standard curve ($y = 0.0009x$; $r^2 = 0.955$).

2.3.2 Total phenolic contents

The test was run for the extracted plant samples to quantify the phenolic contents. Folin-Ciocalteu (F-C) assay was used following the procedure reported by Wolfe et al. [12], and Issa et al. [13], in which the standard curve of Gallic acid was used to calculate the characteristic values as milligram Gallic acid equivalents/grams of the dried plant. The process involved the use of a Gallic acid standard curve ($y = 0.0062x$, $r^2 = 0.987$).

2.3.3. Total flavonoid contents

The contents of flavonoids are articulated as milligram catechin equivalent per gram of the dry weight of the plant. The test was run for the extracted plant samples using aluminum chloride colorimetric assay following the procedure reported by Zhishen et al. [14], using the standard curve of Catechin "secondary metabolite". The total flavonoids were estimated from the following standard curve ($y = 0.0028\ x$, $r^2 = 0.988$).

2.4. DPPH Radical Scavenging Assay

The antioxidant activity of the methanolic extract of *Artemisia monosperma* was assessed using the DPPH free radical scavenging assay following the method of Lim and Quah [15] with minor modifications. A 0.1 mM DPPH solution in methanol was prepared, and 1 mL of this solution was combined with 1 mL of the extract at varying concentrations (50, 100, 200,

300, 400, and 500 mg/mL). The mixtures were shaken thoroughly and incubated in the dark at room temperature for 30 minutes. Absorbance was then recorded at 517 nm using a UV–Vis spectrophotometer, with methanol serving as a blank. Ascorbic acid was used as the standard reference. Radical scavenging activity was expressed as percentage inhibition of DPPH, calculated using the following equation:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Here $A_{control}$ represents the absorbance of the control (DPPH solution without extract), while A_{sample} corresponds to the absorbance in the presence of the extract. The IC_{50} value, defined as the extract concentration required to achieve 50% radical scavenging, was obtained from the dose–response curve. All assays were conducted in triplicate, and the data were reported as mean ± standard deviation.

2.4. Antibacterial activity

2.4.1. Tested organisms

The crude extract of *Artemisia monosperma* was evaluated for antibacterial activity against six pathogenic strains: three Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi*) and three Gram-positive (*Bacillus subtilis, Enterobacter cloacae, and Staphylococcus aureus*). All bacterial isolates were obtained from the Laboratory of Bacteriology, Department of Botany, Faculty of Science, Mansoura University, Egypt.

Antibacterial screening was performed using the agar diffusion method [16]. Sterile filter paper discs (5 mm diameter) were impregnated with the extract at a concentration of 10 mg/mL and placed on nutrient agar plates previously inoculated with 1×10^8 CFU/mL of bacterial suspension. Discs were positioned at the plate center, sealed with Parafilm® (Sigma, St. Louis, MO, USA), and incubated at 37 °C for 24 h. The antibacterial effect was determined by measuring inhibition zones (mm) at three different points around each disc. Penicillin, gentamicin, and chloramphenicol served as positive controls.

3. Results and Discussion

3.1. Qualitative phytochemical screening

The phytochemical screening of *Artemisia monosperma* (Table 1) revealed a wide diversity of secondary metabolites, with flavonoids, phenols, and tannins showing strong presence, while alkaloids, saponins, steroids, glycosides, anthraquinones, and terpenes were detected at moderate levels. Such a profile is consistent with earlier reports on *Artemisia* species, which are recognized for their abundance of bioactive compounds, particularly flavonoids and phenolics, that underpin their pharmacological properties [17, 18].

The predominance of phenols and flavonoids (Table 1) is particularly significant, as these metabolites are powerful antioxidants capable of scavenging reactive oxygen species and protecting biomolecules from oxidative stress. High levels of phenolic constituents in *A. monosperma* have previously been linked to strong antioxidant activities [19,20]. Flavonoids not only contribute to antioxidant defense but also exhibit antimicrobial potential, supporting the use of *Artemisia* extracts in managing infectious diseases [21,22].

The detection of tannins further enhances the plant's bioactivity, since these compounds are known to exert antimicrobial and astringent effects [23]. Likewise, alkaloids and terpenes, though present in moderate amounts, are pharmacologically important. Alkaloids often display antibacterial and cytotoxic activities, whereas terpenes are well-documented for antimicrobial and insecticidal properties [24,25]. The presence of anthraquinones, glycosides, and steroids provides additional therapeutic potential, as these groups are associated with diverse biological functions including laxative, anti-inflammatory, and cardioprotective effects [9]. Overall, the phytochemical richness of *A. monosperma* validates its ethnomedicinal applications and underscores its potential as a natural source of antioxidant and antimicrobial agents.

3.2. Quantitative phytochemical

The quantified levels of phenolics (142.57 ± 2.52 mg GAE/g), flavonoids (46.33 ± 2.10 mg CE/g), and tannins (27.62 ± 1.52 mg TAE/g) in *Artemisia monosperma* highlight its substantial phytochemical richness (Table 2). These elevated concentrations correspond well with previous findings demonstrating that *A. monosperma* typically harbors higher amounts of phenolics, flavonoids, and tannins compared

to other sympatric species such as *Limonium crithmoides*, which in turn is linked to superior antioxidant activity [26]. Moreover, HPLC analyses have identified key phenolic constituents-including gallic acid derivatives-and artemisinin in *A. monosperma*, with quantified artemisinin reaching approximately 1.9 mg/g dry weight [27].

Such abundance in phenolic and flavonoid contents is mechanistically correlated with robust free radical scavenging capabilities; indeed, extracts of *A. monosperma* have demonstrated potent antioxidant activity in DPPH assays, with reported IC_{50} values as low as ~5.48 µg/mL [28]. This suggests that the high levels of these secondary metabolites significantly contribute to their efficacy as an antioxidant agent.

Together, the convergence of high phenolic, flavonoid, and tannin concentrations with compelling antioxidant performance positions *Artemisia monosperma* as a promising candidate for further development into natural therapeutic applications, such as antioxidant formulations or antimicrobial agents.

Table 1. Qualitative phytochemical analysis of some wild plants collected from the coastal desert.

Screening test	Artemisia monosperma
Alkaloids	++
Flavonoids	+++
Phenols	+++
Saponins	++
Tannins	+++
Steroids	++
Glycosides	++
Anthraquinones	++
Terpenes	++

- = absent/trace, + = low, ++ = moderate, +++ = high

Table 2. The concentration of the bioactive secondary chemical constituents of *Artemisia monosperma*.

Samples	Phytochemical Analysis		
	Phenolics	Flavonoids	Tannins
Artemisia monosperma	142.57 ± 2.52	46.33 ± 2.10	27.62 ± 1.52

Phenolics Content "mg gallic acid/1 gm dry extract", Flavonoids Content "mg catechin/1 gm dry extract", Tannins Content "mg tannic acid/1 gm dry extract"

3.3. Antioxidant Activity

The antioxidant potential of *Artemisia monosperma* was clearly demonstrated by its dose-dependent DPPH radical scavenging activity, with inhibition increasing from 10.43% at 5 mg/mL to 75.87% at 50 mg/mL (Table 3). The IC_{50} value of 31.57 mg/L confirms a significant radical scavenging effect, although it was lower than that of the standard ascorbic acid (IC_{50} = 11.77 mg/L). This strong activity can be directly related to the high content of phenolics (142.57 mg GAE/g), flavonoids (46.33 mg CE/g), and tannins (27.62 mg TAE/g) quantified in the crude extract, since these metabolites are widely recognized as primary contributors to antioxidant defense mechanisms [29].

Table 3. Scavenging activity percentage of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and the IC_{50} values of the crude extract of *Artemisia monosperma* and ascorbic acid as standard.

Conc. (mg/ml)	Scavenging activity percentage
	Artemisia monosperma
5	10.43±0.30
10	18.77±0.55
20	31.63±0.90
30	53.41±1.53
40	61.85±1.79
50	75.87±2.16
IC_{50} mg/L	**31.57**
$LSD_{0.05}$	**4.18**
Conc. (mg/ml)	**Ascorbic acid**
1	4.8±0.14
2.5	14.02±0.45
5	40.74±1.16
10	53.28±1.68
15	59.57±1.84
20	72.72±2.27
IC_{50} mg/L	**11.77**
$LSD_{0.05}$	**9.75*** **

The high phenolic content of *A. monosperma* aquatic extract is consistent with reports from other *Artemisia* species, which are often characterized by abundant polyphenols and associated strong antioxidant activity [26]. Flavonoids are particularly important because of their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals, while tannins can form complexes with pro-oxidant metals and suppress oxidative damage [28]. Such a phytochemical profile suggests that the antioxidant activity of *A. monosperma*

is not only due to individual compounds but also to possible synergistic interactions among its different bioactive metabolites.

The ecological adaptation of desert plants to harsh environments may also explain the accumulation of high levels of phenolic and flavonoid compounds, which serve as protective agents against oxidative stress induced by strong sunlight, drought, and salinity [30]. From a pharmacological perspective, the combined evidence of abundant phytochemicals and notable radical scavenging activity positions *Artemisia monosperma* as a promising candidate for development into natural antioxidant formulations that may be used to prevent oxidative stress-related disorders.

3.3. Antibacterial activity

The evaluation of the antibacterial activity of the methanol extract from *A. monosperma* against a panel of clinically significant bacteria reveals a potent and broad-spectrum antimicrobial profile. Its efficacy is notably competitive with several standard antibiotics, highlighting the significant potential of plant-derived crude extracts as sources of novel antibacterial compounds, especially in the face of escalating antimicrobial resistance (AMR) (Table 4).

A particularly compelling finding is the extract's robust performance against Gram-negative pathogens, which are notoriously difficult to treat due to their impermeable outer membrane and efficient efflux pumps [31]. The activity of the *A. monosperma* extract against *Escherichia coli* (21.91 mm) was comparable to ampicillin (20.58 mm) and tetracycline (21.6 mm), and significantly surpassed chloramphenicol (10.81 mm) (Table 4). This suggests the presence of secondary metabolites in the extract capable of penetrating or disrupting the complex Gram-negative cell envelope.

Table 4. Antibacterial activity of the essential oil extracted from selected plant and some selected reference antibiotics.

extract (10 mg/ml)	Gram-negative bacteria			Gram-positive bacteria		$LSD_{0.05}$
	Escherichia coli	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Bacillus subtilis***	***Staphylococcus aureus***	
A. monosperma	21.91	14.76	10.74	19.71	17.4	1.05***
Standard antibiotic (10 mg/ml)						
Ampicillin	20.58	6.02	0	8.05	28.48	1.06***
Chloramphenicol	10.81	10.14	0	19.65	14.59	1.21***
Gentamicin	25.43	10.88	0	20.18	23.57	1.04***
Tetracycline	21.6	0	10.4	10.86	18.17	2.31***

The most significant result is against *Salmonella typhimurium*. The crude extract produced a clear zone of inhibition (10.74 mm), while three first-line antibiotics—ampicillin, chloramphenicol, and gentamicin—showed no activity (0 mm) (Table 4). This indicates a multi-drug resistant (MDR) phenotype in the tested strain, a grave concern in public health [32]. The ability of the *A. monosperma* extract to inhibit this resistant strain underscores its value as a promising source of anti-infective agents that could circumvent common resistance mechanisms. Its efficacy was on par with tetracycline (10.4 mm), which remained effective.

Against *Pseudomonas aeruginosa*, a champion of intrinsic resistance, the extract (14.76 mm) demonstrated markedly stronger activity than ampicillin (6.02 mm) and was comparable to chloramphenicol (10.14 mm) and gentamicin (10.88 mm). The complex mixture of compounds in a crude extract, potentially including alkaloids, flavonoids, tannins, and terpenoids, may act synergistically on multiple cellular targets, such as membranes, enzymes, and genetic material, making it difficult for bacteria to develop resistance [33, 34]. This multi-target action is a key advantage over single-target synthetic antibiotics.

The extract also exhibited strong activity against the Gram-positive bacteria, *Bacillus subtilis* (19.71 mm) and *Staphylococcus aureus* (17.4 mm). Its effect on *B. subtilis* was nearly identical to chloramphenicol (19.65 mm) and gentamicin (20.18 mm) and far superior to ampicillin and tetracycline. The activity against *S. aureus* was greater than chloramphenicol.

The susceptibility of Gram-positive bacteria is often higher due to the absence of a protective outer membrane, allowing the bioactive constituents of the extract to more easily reach the cell wall and membrane [35]. The very low Least Significant Difference (LSD) values confirm that the differences in antibacterial activity between the treatments are statistically robust and reliable.

4. Conclusion

In conclusion, this study provides a thorough scientific validation of the traditional use of *Artemisia monosperma*. Comprehensive phytochemical profiling revealed that the methanolic extract is rich in potent bioactive compounds, particularly phenolics, flavonoids, and tannins. This chemical richness directly correlates with the observed significant biological activities. The extract demonstrated considerable antioxidant potential, effectively scavenging DPPH free radicals in a dose-dependent manner. This activity, quantified by an IC_{50} value of 31.57 mg/L, can be confidently attributed to its high concentration of antioxidant metabolites, which serve as hydrogen donors and radical stabilizers. More importantly, the investigation revealed potent, broad-spectrum antibacterial efficacy. The extract's performance was notably exceptional against multi-drug resistant (MDR) strains, particularly *Salmonella typhimurium*, where it outperformed several first-line antibiotics. Its effectiveness against other challenging pathogens like Pseudomonas aeruginosa and its strong activity against Gram-positive bacteria further underscore its potential. This broad-spectrum activity is likely due to the synergistic action of its complex mixture of phytochemicals, which can target multiple microbial cellular structures and functions simultaneously, thereby reducing the likelihood of resistance development. Therefore, the findings from this study firmly establish *Artemisia monosperma* as a promising and valuable natural resource.

5. References

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